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A METHOD FOR THE STAINING OF NEGRI BODIES.*[1]

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SINCE the description by Negri in 1903 of certain bodies found in the brains of animals dying of rabies, many methods have been recommended for the staining of these structures. This is in itself evidence that all of these methods are more or less unsatisfactory at times. Although in most cases the Negri bodies are numerous, large, and easily recognized, investigators are agreed that in some cases this recognition is very difficult and uncertain.

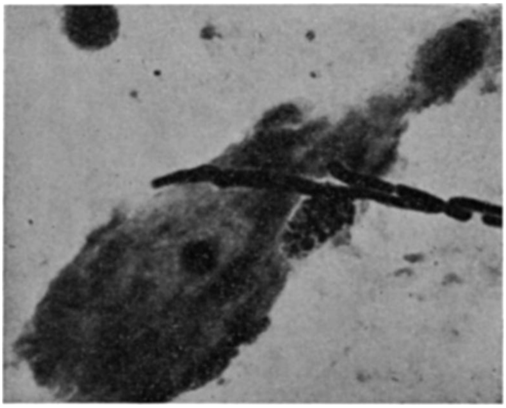

FIG. 1.—Photo-micrograph from cornu ammonis of a dog made three days after death. (Post mortem bacterial invasion.) Negri bodies lying within nerve cells. ×1,000.

During the past 18 months dogs suspected of being rabic, including those killed by the police, have been sent to the laboratory of the city bacteriologist of St. Louis, for diagnosis. In the beginning of this work we were occasionally unable to demonstrate the presence of Negri bodies in animals which were proven to be rabic through inoculation tests. In the past year we have developed an eosin methylene blue stain which has proven so satisfactory and is so rapid and simple that it seems desirable to report the method.

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* Received for publication October 29, 1908.

[1] Read before the Laboratory Section of the American Public Health Association, Winnipeg, Canada, August 26, 1908

In the routine examination smears between two glass slides are made from the cornu ammonis, cerebellum, and cerebral cortex. Two slides are preferable to one slide and one cover glass, as the thickness of the smear can be controlled more easily and there is less danger of soiling the hands. The smear is to be treated as follows:

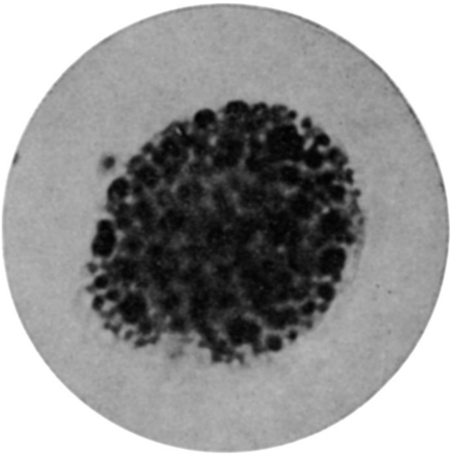


FIG. 2.—Photo-micrograph of Negri body in a smear from the cornu ammonis of a dog. ×3.000.

Fix in methyl alcohol one minute.

Wash briefly in water to remove the alcohol.

Immerse in an old saturated solution (95 per cent alcohol) of alcohol soluble eosin from one to three minutes.

Wash two or three seconds in water to remove the excess of eosin.

Immerse in a fresh solution of Unna's alkaline methylene blue 5 to 15 seconds.

Wash briefly in water.

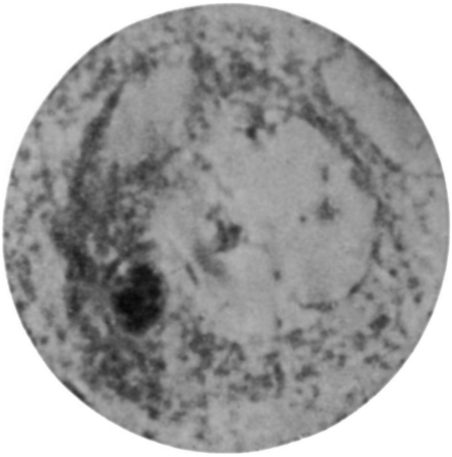


FIG. 3.—Photo-micrograph of a section from the cornu ammonis of dog, showing a Negri body lying within the nerve cell. ×3,000.

Decolorize in 95 per cent alcohol; and follow with absolute alcohol, xylol, and balsam, or blot and dry in the air.

The entire process requires less than five minutes.

The structure of the bodies is more sharply defined if the smear is not allowed to dry before being fixed and stained. Smears which have dried for several days or weeks cannot be stained satisfactorily.

The older the saturated solution of eosin (alcoholic), the more rapidly and intensely it stains. A solution less than two months old will not yield good results.

The methylene blue (Unna's) will produce a very disturbing precipitate if it be older than a week or two. The fresher the solution, the more sharply defined are the "inner" bodies.

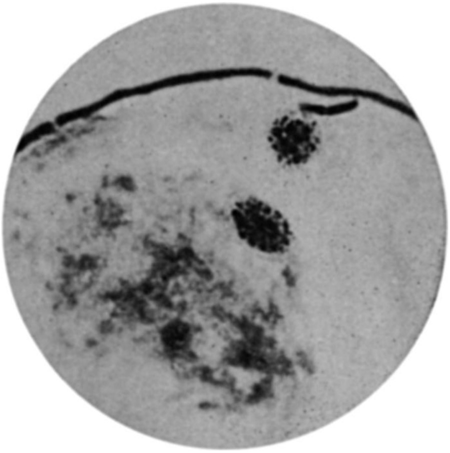


FIG. 4.—Photo-micrograph from cornu ammonis of a dog made three days after death. (Postmortem bacerial invasion.) Negri bodies lying within nerve cells. ×1,000.

During the decolorization the slides should be examined from time to time. Decolorize until only the nerve cells are blue and the red cells have become bright red. It has been our experience that when the red blood cells appear red, the nucleoli of the nerve cells a

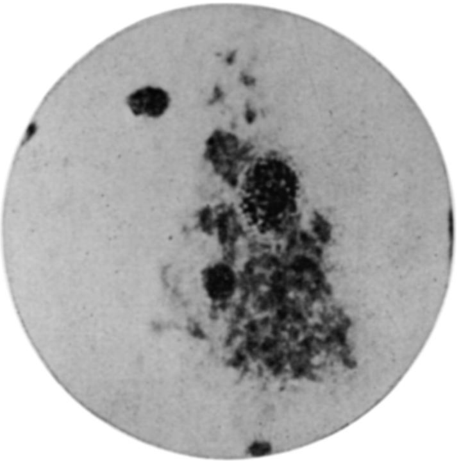
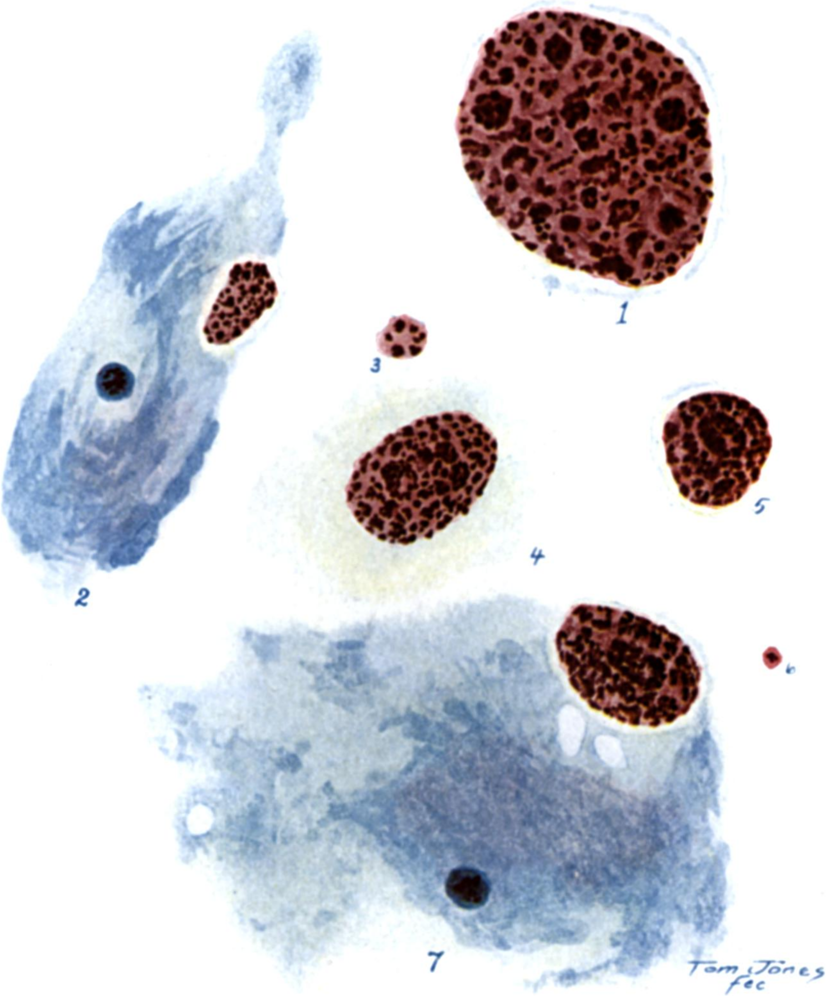


FIG. 5.—Photo-micrograph from cornu ammonis of a dog made three days after death. (Postmorlem bacterial invasion.) Negri bodies lying within nerve cells. ×1,000.

PLATE 5.



deep blue, and the protoplasm a pale blue, the Negri bodies will be seen colored a light red with sharply defined dark bluish-red "inner" bodies. It is better to decolorize too much than too little. The results will be more uniform if the staining is done in staining-dishes or jars.

The advantages of this method are:

Strong contrast between Negri bodies, nerve cells, and blood cells; the absence of a granular precipitate, so disturbing in many methods as to render the recognition of the small-sized bodies impossible; rapidity and simplicity.

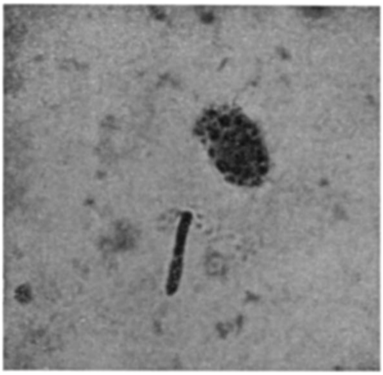


FIG. 6.—Photo-micrograph of Negri body from cornu ammonis of dog. Smear. ×1,000.

In the staining of material fixed in Zenker's fluid or acetone, the sections should be stained three to five minutes in the eosin and 30 seconds to two minutes in the methylene blue. In sections the inner bodies appear a deep blue.

A little practice and familiarity with the solutions employed will enable one to judge correctly the time necessary for each step.

DESCRIPTION OF PLATE 5.

Drawings showing the Negri bodies in smears from the cornu ammonis of a dog. (Bacteria omitted.)

Fig. 1 corresponds to photo-micrograph 2. × 3,000.

Fig. 2 corresponds to photo-micrograph 1. × 1,000.

Figs. 5 and 7 correspond to photo-micrograph 4. × 3,000.